Chapter 16

Selected Endothelial Responses after Ionizing Radiation Exposure

Bjorn Baselet, Raghda Ramadan,
Abderrafi Mohammed Benotmane,
Pierre Sonveaux, Sarah Baatout and An Aerts

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http://dx.doi.org/10.5772/intechopen.72386

Abstract

Along with the development of novel chemotherapeutic agents, radiation therapy has revolutionized the prognosis of patients with various cancers. However, with a longer life expectancy, radiation treatment-related comorbidity, like cardiovascular diseases (CVDs), becomes an issue for cancer survivors. In addition, exposure to X-rays for medical diagnostics is dramatically increasing at the present times. A pressing question is whether or not exposure to these very low doses can cause health damage. Below 0.5 gray (Gy), an increased risk cannot be evidenced by epidemiology alone, and in vitro and in vivo mechanistic studies focused on the elucidation of molecular signaling pathways are needed. Given the critical role of the endothelium in normal vascular functions, a complete understanding of radiation-induced endothelial dysfunction is crucial. In this way, the current radiation protection system could be refined if needed, making it possible to more accurately assess the cardiovascular risk in the low-dose region. Finally, radiation-induced CVD, like CVD in general, is a progressive disorder that may take years to decades to manifest. Therefore, experimental studies are warranted to fulfill the urgent need to identify noninvasive biomarkers for an early detection and potential interventions—together with a healthy lifestyle—that may prevent or mitigate these adverse effects.

Keywords: ionizing radiation, radiation therapy, X-ray diagnostics, cardiovascular disease, atherosclerosis, endothelial dysfunction, inflammation, DNA damage, apoptosis, cell cycle, oxidative stress, mitochondrial dysfunction and metabolic changes, premature senescence, intercellular communication

1. Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the Western world. It accounts for nearly one-third of all deaths worldwide. There are multiple contributory risk factors for heart disease. Some are of a controllable nature, such as lifestyle, dietary factors, and metabolic disorders, such as high cholesterol levels and hypertension. Others are noncontrollable risk factors, such as gender, age, and genetic predisposition [1, 2]. In addition, there are environmental factors affecting the risk of CVD, ionizing radiation being one such factor.

It has been known for a long time that high doses of radiation, such as those delivered during radiotherapy, cause damage to the heart and vasculature and thus increase the risk of CVD. Data from animal experiments have strongly supported this observation [3–6]. However, for doses <0.5 gray (Gy), epidemiological data are suggestive rather than persuasive. Therefore, the magnitude of CVD risk in the low-dose region where issues of radiation protection usually operate is not clear [3–6].

Various issues, such as occupational radiation exposure, future of nuclear power, manned space flights, and threat of radiological terrorism, call for a thorough understanding of low-dose health risks [7]. The main concern is, however, an increasing use of ionizing radiation for diagnostic medical purposes (**Figure 1**) [8]. For instance, since 1993, the number of computed tomography (CT) scans has increased four times in the United States, and a similar trend is observed in Europe [9]. Medical radiation is the largest source of radiation exposure in Western countries, accounting for a mean effective dose of 3.0 millisievert (mSv) on average per capita per year from diagnostic procedures only, corresponding to a radiological risk of 30 chest X-rays [10]. Of note, doses from therapeutic procedures are not taken into account in this number. Although the health benefits of these improved diagnostic procedures are huge, concerns are raised regarding "overuse" and potential associated health risks [11].

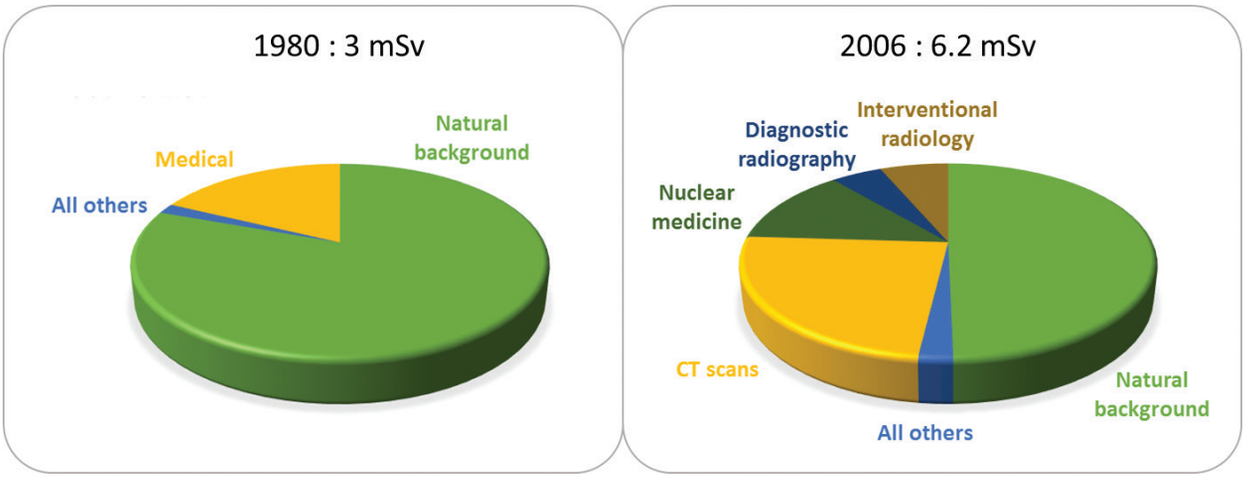

Figure 1. Average annual effective dose per person received in 1980 (left panel) and in 2006 (right panel) in the United States. The large increase in the use of ionizing radiation for medical purposes, in the period 1980–2006, contributed to a total increase from 3.0 mSv in 1980 to 6.2 mSv in 2006. Similar trends are observed in other industrialized countries [1].

1.1. What is ionizing radiation?

From natural to manufactured sources, life on earth is exposed on a daily basis to ionizing radiation. Defined as a type of energy released by atoms that travel in the form of electromagnetic or particles, this energy can eject tightly bound electrons from the orbit of an atom, causing the atom to become ionized [12]. In nature, one can distinguish three main types of ionizing radiation: alpha (α), beta (β) particles, and gamma (γ) rays. They are all produced by naturally occurring substances with unstable nuclei (e.g., cobalt-60 and cesium-137) that spontaneously undergo radioactive decay. During the decay process, energy is lost via emission of ionizing radiation in the form of electromagnetic γ-rays and/or charged particles (e.g., α- and β-particles) [13]. One of the most common manufactured forms of ionizing radiation is X-ray radiation. X-rays are in most aspects similar to γ-rays but differ in origin. While γ-rays are derived from the natural decay of radioactive elements, X-rays are artificially produced in X-ray generators by directing a stream of high-speed electrons at a target material, such as gold or tungsten [14]. When electrons interact with atomic particles of the target, X-radiation is produced [12]. In addition to the most common forms listed above, there are many other forms of ionizing radiation of human or natural origin. Examples are neutrons, accelerated ions and fission fragments [15, 16]. These less common forms can have different biological effects, which can be exploited, for example, in hadron therapy for cancer treatment [17].

1.2. Radiation metrics

Biological effects of ionizing radiation are related to energy deposition in matter. To assess the impact of ionizing radiation on human health and to set guidelines in radioprotection, units to measure a dose and its biological effects are required.

The absorbed dose is defined as the amount of energy, originating from any type of ionizing radiation and any irradiation geometry that is absorbed per unit mass of material. The international SI unit for absorbed dose is gray (Gy). One Gy represents the absorption of 1 joule of energy in 1 kilogram of mass (1 J/kg). This definition is pure physical, as it does not consider the quality of the ionizing radiation type and the extent of biological damage it inflicts to certain tissues and/or organs. As a result, the terms equivalent dose and effective dose have been introduced [18].

The equivalent dose takes into account the ability of a particular kind of ionizing radiation to cause damage. It is obtained by multiplying the absorbed dose (Gy) with a radiation-weighting factor (wR) attributed to each different radiation type (e.g., the wR of photons and electrons is 1, the wR of protons and charged ions is 2, and the wR of α particles and fission fragments is 20). The international SI unit for equivalent dose is the sievert (Sv) [18].

The effective dose is defined as the weighted sum of all tissue and organ equivalent doses multiplied by their respective tissue-weighting factor (wT). It expresses the biological effect that a certain type of ionizing radiation has on the human body. wT values have been defined to represent the contributions of individual organs and tissues to overall radiation effects on the human body. Similar to the equivalent dose, the effective dose has sievert (Sv) as international SI unit. Care should be taken with wT values because they constitute an average

over both genders and adult ages to reflect the radiation burden to an average human adult [18, 19]. Examples of effective doses associated with different sources of ionizing radiation are presented in **Table 1**.

1.3. Protection against radiation exposure

Short after the discovery of ionizing radiation by Röntgen in 1895, its detrimental effects became apparent, and people tried to protect themselves [24]. Nowadays, the International Committee on Radiological Protection (ICRP) and the US National Council on Radiation Protection and Measurements (NCRP) aim to protect people by advising means for achieving this, e.g., regulatory and guidance limits [18, 25].

The major question that keeps radiation protection bodies busy and that became the foundation of radiation protection guidelines worldwide is "How much is harmful?" This question is particularly relevant for low-dose exposures for which health impact is not yet fully elucidated. Although a large number of epidemiological and radiobiological studies have been performed to date in order to investigate the effects of low doses of ionizing radiation [26–47], accurate risk assessment is not yet available [18]. Current guidelines for protection against low-dose radiation are based on cancer risk estimates from epidemiological studies. As discussed further, cohorts include atomic bomb survivors, occupationally exposed people, patients (diagnostics or therapeutics), and environmentally exposed people [48]. In general, an excess cancer

Source	Effective dose (mSv)*
Dental X-ray	0.005
Radiography of the chest	0.1
One return flight (New York-London)	0.1
Radiography of the abdomen	1.2
CT of the head	2
Natural background (per year)	2.4
Mammography	3
CT of the chest	7
CT of the abdomen	6–10
CT of the pelvis	8–10
Coronary CT angiography	12
Myocardial perfusion study	10–29
Myocardial viability study	14–41
Annual occupational dose limit	20
Radiotherapy (delivered in fractions)	40,000–70,000

*Doses are whole-body doses, except those of medical exposure, which are delivered to a specific organ. CT, computed tomography; Sv sievert [7, 19–23]

Table 1. Representative effective doses associated with different sources of ionizing radiation.

risk can be statistically evidenced for doses above 100 mGy. Nevertheless, doses below 100 mGy are inconclusive due to two practical limits of epidemiological studies: low statistical power that generates random errors and demography that gives rise to systematic errors. Due to a high natural incidence of cancer, a lifetime follow-up of larger cohorts would be needed to quantify excess cancer risks due to a low dose of ionizing radiation. This is practically infeasible. Furthermore, confounding factors, such as lifestyle risk factors for CVD, can hamper accuracy to confidently detect a small increase in cancer mortality (discussed in Section 2.4). Any inadequacy in matching between control and study groups may give rise to a bias that cannot be merely reduced by expanding the size of the groups [49]. As a consequence, risk assessment in the low-dose region (<100 mGy) is based on extrapolations made from high-dose risk estimates [50]. For cancer, it is widely accepted that the tumorigenic risk increases with radiation dose without the presence of a threshold (the stochastic linear non-threshold [LNT] model). This assumption implies that no dose is absolutely safe, resulting in implementation of the "as low as reasonably achievable" principle [51, 52].

In contrast to cancer, non-cancer diseases have for long not been considered as health risks following exposure to low doses of ionizing radiation. Consequently, they were believed to have a threshold dose below which no significant adverse risks are induced (deterministic linear threshold model) [18, 53]. This idea has been challenged by epidemiological findings showing an excess risk of non-cancer diseases following exposure to doses lower than previously thought [34, 54]. Epidemiological evidence suggests an excess risk of CVD mortality above 0.5 Gy [34, 54]. For doses below 0.5 Gy, the dose-risk relationship is still unclear. However, if the relationship proves to be without a threshold, this may have a considerable impact on the current radiation protection system, since the overall excess mortality risk following low-dose exposure could double [55].

2. Radiation-induced cardiovascular disease

2.1. Epidemiology of radiation-induced CVD

Current predictions indicate that in Western countries almost one of three people will develop cancer during their lifetime [56]. About 50–60% of all cancer patients will undergo radiotherapy with radiation doses averaging 1.8–2 Gy per fraction [57]. During the radiotherapeutic treatment of tumors located in the mediastinal region of the human body (breast, lung, and esophageal cancers), the heart and its blood vessels incidentally receive a part of the radiation dose [46]. Exposure of the cardiovascular system to these therapeutic doses is known to be associated with CVDs. The first epidemiological evidence of this association came from radiation-treated Hodgkin's lymphoma survivors in the 1960s. In a study of 258 Hodgkin's disease patients followed for a median of 14.2 years (range 0.7–26.2) after radiotherapy, cumulative risk for ischemic event increased from 6.4% (95% confidence interval (CI), 3.8 ± 10.7) at 10 years to 21.2% (95% CI, 15 ± 30) at 20–25 years after radiotherapy treatment. Risk for myocardial infarction was 3.4% (95% CI, 1.6 ± 7.0) at 10 years and 14.2% (95% CI, 9 ± 22) at 20–25 years, and risk for ischemic cardiac mortality was 2.6% (95% CI, 1.1 ± 6.1) at 10 years and 10.2% (95% CI, 5.3 ± 19) at 25 years (**Figure 2A**) [58]. Cardiac fibrosis, which causes cardiac dysfunction, arrhythmias, and heart failure, is also seen in Hodgkin's lymphoma survivors but is rather the result of the use of

anthracyclines [59]. Radiation-induced cardiovascular disorders are based rather on the damage to the blood vessels. Later, in the study of Darby et al., 2168 breast cancer patients were followed between 5 and more than 20 years after radiotherapy. It was found that women irradiated for left breast cancer (estimated mean heart dose 6.6 Gy) had higher rates of major coronary events than women irradiated for right breast cancer (estimated mean heart dose 2.9 Gy; P = 0.002). Excess relative risk (ERR), a measure that quantifies how much the level of risk among persons with a given level of exposure exceeds the risk of nonexposed persons [60] for major coronary events was 7.4% per Gy (95% CI, 2.9–14.5) when all follow-up times and all breast cancer patients were included (**Figure 2B**) [54].

Additional proofs of increased risk of CVDs after high-dose exposure were provided during the follow-up of Japanese atomic bomb survivors. During a 53-year follow-up of 86,611 members of the Life Span Study cohort, excess relative risk of death from heart disease per Gy was 0.14 (95% CI 0.06–0.23) (**Figure 2C**) [34]. Although there is a large number of epidemiological studies showing a clear excess of CVD risk above 0.5 Gy, they are of limited use for quantitative risk assessment, because individual dosimetry has yet to be performed [35]. In addition, even if an adverse effect can be evidenced at relatively high doses of ionizing radiation, mechanisms by which therapeutic doses affect the cardiovascular system are still not completely understood [28].

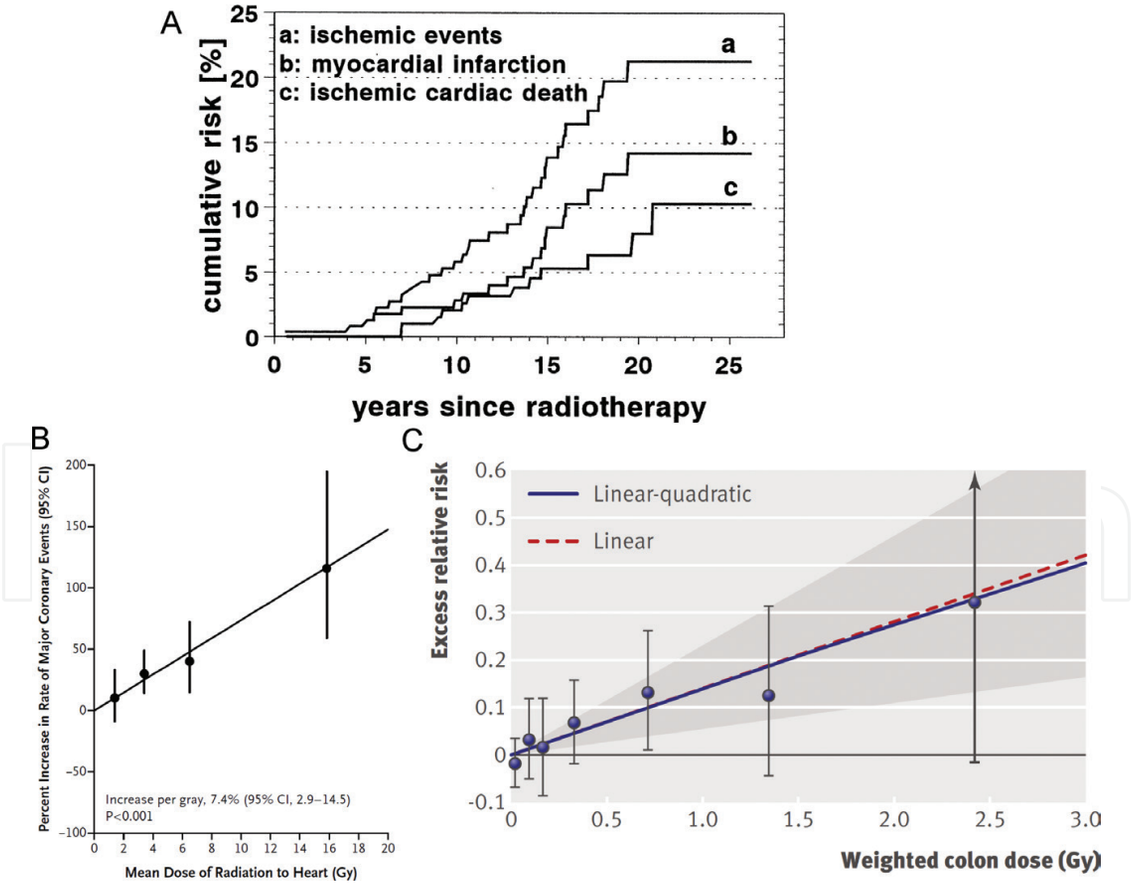

Figure 2. Epidemiological evidence for an increased risk of CVDs after exposure to ionizing radiation. (A) Cumulative risk curves for the occurrence of cardiac events in Hodgkin's lymphoma survivors [58]. (B) Rate of major coronary events according to mean radiation dose to the heart given during breast cancer radiotherapy, as compared with the estimated rate with no radiation exposure to the heart [54]. (C) Excess relative risk for death from heart disease in Japanese atomic bomb survivors. Shaded area is the 95% confidence region for the fitted lines [34].

When the heart receives a radiation dose lower than 0.5 Gy, epidemiological evidence is less strong than that for higher doses. The most informative cohort in this respect is composed of Japanese atomic bomb survivors. It is of high value for low-dose epidemiology as a source for risk estimation due to its large size, the presence of both sexes and all ages, and because irradiated people have well-characterized individual dose estimates [36]. Studies in occupationally exposed individuals are also of interest as they generally involve relatively low doses received during repeated exposures. Examples of such cohorts are nuclear industry workers from 15 countries (the 15-country study) [37], the UK National Registry for Radiation Workers [38], the National Dose Registry of Canada [39], the Chernobyl liquidator cohort [40], and the Mayak cohort [41–43]. The last cohort is composed of workers from Mayak PA, the first and largest Russian nuclear factory for plutonium production where the majority of workers were exposed to ionizing radiation during the first period of operation [61]. In addition, data can also be acquired from environmentally exposed groups, such as settlements located at the vicinity of the Techa River [44] and the Semipalatinsk nuclear test area [45].

When taking into account all epidemiological data on CVD effects of ionizing radiation, a small but highly statistically significant ERR of 0.09 per Gy (95% CI, 0.07–0.12) was observed at doses higher than 0.5 Gy [35]. In addition, ERR of CVD mortality was estimated at 0.08 (95% CI, 0.04–0.12). In other words, receiving 1 Gy of ionizing radiation to the heart and its blood vessels increases the risk of CVD mortality with 8% in comparison to nonexposed people. This assumed risk is rather large and may therefore have serious implications for public health. Indeed, considering the high background rate of CVD, the absolute number of excess cases could be substantial [62]. In order to find an association between low-level radiation exposure and CVD risk in a general unselected population, this meta-analysis was extended by Little et al. [55]. When taking into account 717,660 individuals from the Japanese atomic bomb survivor and occupational and environmental exposure studies listed above, a statistically significant ERR coefficient of 0.10 (95% CI, 0.05–0.15) for coronary artery disease was observed as a result of exposure to low-level radiation more than 5 years prior to death [55]. A linear association between ERR and radiation dose was assumed even in the low-dose range, because there was little evidence of nonlinearity in the dose-response curves for CVD in Japanese atomic bomb survivors [34, 63] and in Mayak workers [41]. Authors further argued that the consistency of ERR/Gy between Japanese atomic bomb survivors with moderate radiation doses [34, 63] and occupational cohorts with low doses could be used to support the notion of a linear relationship between ERR of CVD mortality and low doses of ionizing radiation [55]. In a recent third analysis of the Life Span Study cohort of atomic bomb survivors with 105,444 subjects, the shape of the dose-response curve for solid cancer incidence was found significantly different among males and females ($P = 0.02$). For females, dose-response was consistent with linearity, but for males dose-response best fitted a linear-quadratic model [64]. If this were to be confirmed, the overall excess risk of CVD-associated mortality after exposure to low doses of radiation would be about twice that associated to radiation-induced cancers, which ranges from 4.2% to 5.6% per Sv for the cohort populations discussed above [55, 65] and would even be different between both sexes.

2.2. Pathophysiology of radiation-induced CVD

Following radiotherapy of the thoracic part of the human body for mediastinal lymphoma, breast, lung, and esophageal cancers, the heart incidentally receives a part of the therapeutic dose [46].

As indicated in the epidemiology section, high-dose radiation exposure of the heart and its vessels is associated with a risk of radiation-induced CVD [34, 54, 55]. In this context, coronary artery disease is considered to be the major cardiovascular complication [28, 30, 54]. Two studies provide molecular and cellular mechanisms accounting for increased morbidity and mortality of coronary artery disease following radiation exposure. First, radiation can influence the pathogenesis of age-related atherosclerosis, thereby accelerating the development of atherosclerosis in coronary arteries [28]. Growing atherosclerotic plaques narrow the blood vessel and hamper the blood stream (**Figure 3**). Second, damage to the heart microvasculature can reduce blood flow to the myocardium, causing myocardial ischemia, which promotes acute infarction [30]. Because endothelial activation and dysfunction are major causes of atherosclerosis, much of the current radiobiological research is exploring the molecular and phenotypic effects of ionizing radiation in endothelial cells in the context of radiation-induced CVD [66, 67]. It should be noted, however, that there are also other clinical manifestations of radiation-related CVD, such as pericarditis, congestive heart failure, and heart fibrosis [30, 68, 69]. Radiation-induced pericarditis is caused by damage to the cardiac microvascular network in combination with fibrosis of cardiac venous and lymphatic channels. This ultimately leads to accumulation of a fibrin-rich exudate in the pericardium, causing pericardial tamponade. Congestive heart failure is attributed to radiation-induced fibrosis of the myocardium, which ultimately leads to decreased elasticity and extensibility of cardiac walls, thereby reducing the ejection fraction [70]. To learn more about putative mechanisms, the interested reader is referred to some excellent recent reviews [69, 71].

2.3. Gaps in the current knowledge of radiation-induced CVD

Available epidemiological studies have limited statistical power to detect a possible excess risk of CVD following exposure to radiation doses lower than 0.5 Gy. Limited power is due both to the high background level of CVD in studied populations and to the existence of many

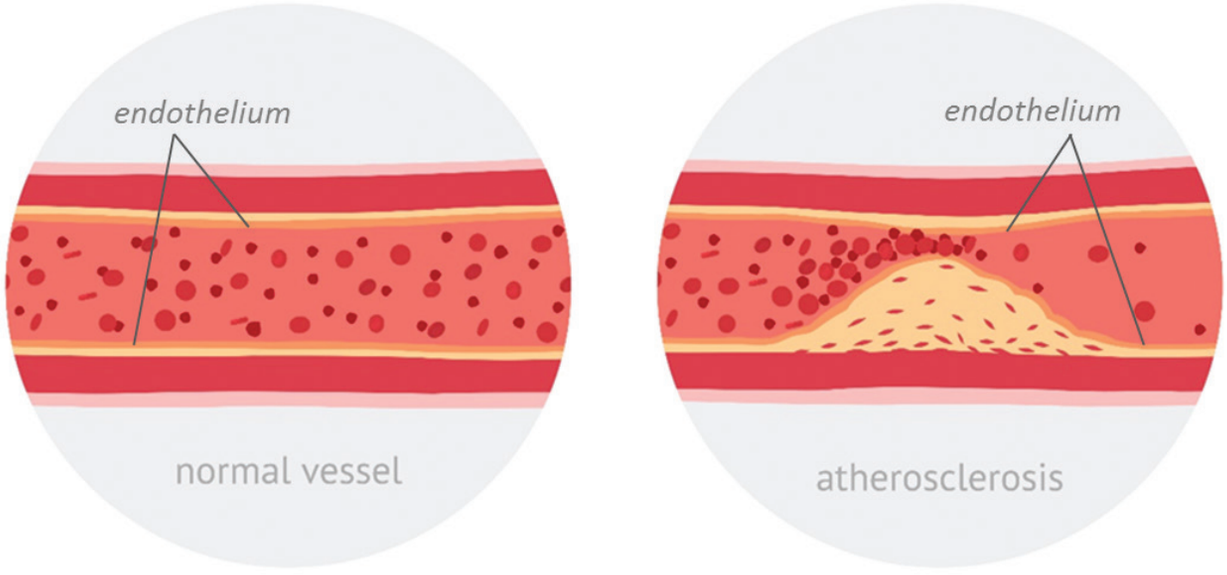

Figure 3. Longitudinal cut of a normal, healthy blood vessel (left) and of a blood vessel with an atherosclerotic plaque hampering the blood stream (right). Damage to the endothelium is an important trigger of atherosclerosis, itself a main cause of CVD.

potentially confounding risk factors. For example, occupational studies have to deal with the "healthy worker" effect, and the study of A-bomb survivors has to deal with the "healthy survivor" effect. Both selection effects occur when healthy individuals with lower mortality and morbidity rates are selectively retained at a specific site (work and living area, respectively) where they accumulate higher doses and therefore confound the dose-risk relationship [37]. Other potential confounders in epidemiological studies are lifestyle risk factors for CVD (e.g., smoking, alcohol consumption, obesity, diabetes, hypertension) [35, 55] prognosis of cancer treatment regimens [30], distribution of the dose range, accuracy of dosimetry, duration of follow-up after exposure, and correct assignment of the cause of mortality [62]. For these reasons, the number of people needed to quantify the excess risk of a dose <0.5 Gy is unfeasibly high. In the context of radiation-related cancer, for example, a cohort of 5 million people would be needed to quantify the excess risk of a 10 mGy dose, assuming that the excess risk is in proportion to the dose [7]. Moreover, CVD may occur a long time after exposure to doses below 30 Gy (approx. 10–30 year lag) [30, 72, 73]. As a result, a long follow-up period of time is needed to determine the nature and magnitude of risks following individual exposure to lower doses.

Despite the fact that epidemiological studies have led to significant insights in radiation-related CVD risk, there are still many uncertainties that need to be addressed. Does CVD risk occur only above a specific radiation dose? Is the latency of CVD development dependent on the dose? Which are the sensitive targets in the heart and vasculature (e.g., fibroblasts, vascular smooth muscle cells, and endothelial cells)? Does radiation exposure affect CVD incidence or progression or both? Is there a difference between single dose and fractionated and chronic exposure? How does the time interval between two consecutive dose fractionations play a role in the induction of damage? These questions need to be answered to provide a more accurate dose risk assessment in order to improve the current radiation protection system.

Classical epidemiological studies are not adapted to provide answers to these questions. There is, therefore, a clear need for more detailed epidemiological studies that would be capable of addressing potential confounding factors and selection biases that could influence results. Furthermore, there is a particular need for a better understanding of the biological and molecular mechanisms responsible for the association between ionizing radiation and CVD [6]. Hence, a more directed approach is required, such as molecular epidemiology that integrates epidemiology and biology [55]. Radiobiological research is thus essential for understanding the radiation-related CVD risks, both at high and low doses. In other words, accurate risk estimation will be possible only based on comprehensive biological and molecular understanding of what ionizing radiation does to the cardiovascular system. To date, the induction of radiation-related CVD risks is believed to be the result of endothelial dysfunction, which will be discussed in the next section [30].

3. Endothelial cell responses after ionizing radiation exposure

The endothelium could be a critical target in ionizing radiation-related CVD [74]. The endothelium is a single layer of cells that lines the interior of the vascular system and of the heart and has thus a strategic position between the blood and the surrounding tissues. It is a highly active organ system that is constantly sensing and responding to changes of the extracellular environment to maintain a normal function of the vascular system [75].

Endothelial cells are involved in a wide range of physiological processes, such as regulation of vascular tone, vascular permeability, blood coagulation/fibrinolysis, and inflammation, which are needed to maintain proper vascular functioning (**Figure 4**) [76]. Endothelial dysfunction has been observed in patients with atherosclerosis and in patients that exhibit CVD risk factors such as smoking, dyslipidemia, obesity, and diabetes mellitus [77] and is considered to be one of the first predictive indicators of cardiovascular morbidity and mortality [78].

A dysfunctional endothelium is characterized by inflammation, DNA damage, oxidative stress, alterations of coagulation and platelet pathways, senescence, and cell death, all of which are observed after radiation doses above 1–2 Gy, as shown in many in vitro and in vivo studies [6, 28, 79–81]. Comparatively, both protective and detrimental effects have been reported for low-dose exposure, suggesting that multiple mechanisms may influence radiation-induced atherosclerosis [6, 62]. Increasing evidence also suggests a role of intercellular communication in the endothelial cell response to ionizing radiation [82]. All of these endpoints are briefly discussed in the following paragraphs. In addition, other pathological effects of ionizing radiation on the endothelium are observed like impaired endothelial regulation of vascular tone [83–87], loss of the endothelial monolayer integrity [88–92], and procoagulant and prothrombic conditions [28, 93–108].

3.1. Inflammation

Endothelial expression of adhesion molecules plays an important role in recruiting inflammatory cells from the bloodstream into the vessel intima where they transform into foam cells, elements

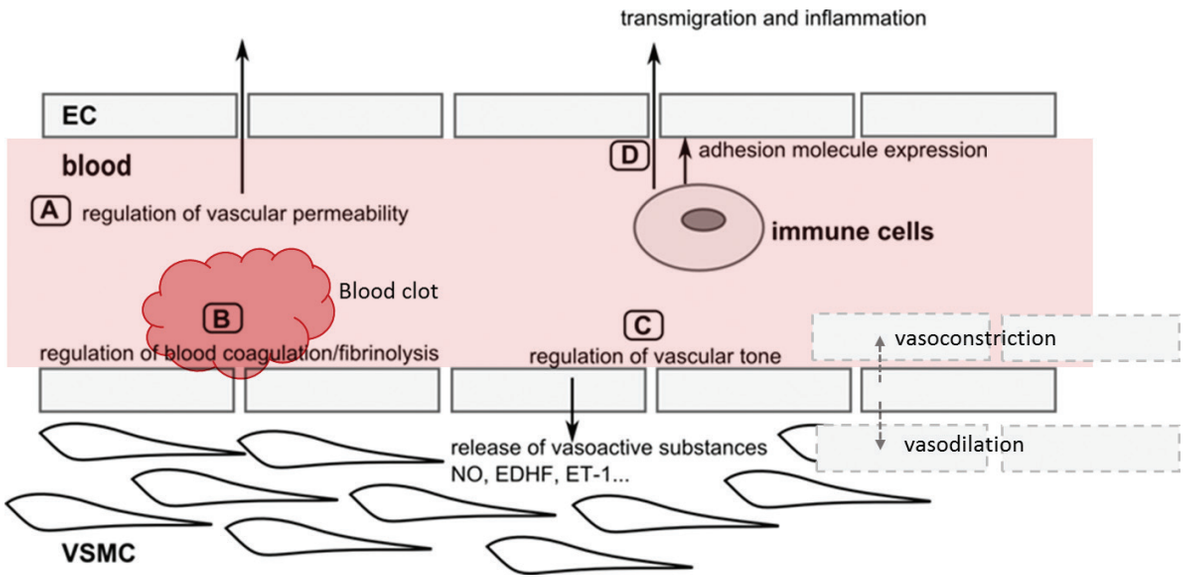

Figure 4. Overview of the major physiological functions of the arterial endothelium. (A) The endothelium (ECs, endothelial cells) forms a selective barrier regulating solute flux and fluid permeability between the blood and surrounding tissues [78]. (B) Formation of a thrombus or blood clot is referred to as intravascular coagulation, and the breakdown of a thrombus is referred to as fibrinolysis. Normal endothelium has antithrombotic and profibrinolytic properties and actively represses platelet adhesion and aggregation. Vessel damage or exposure to pro-inflammatory molecules shifts the balance toward more prothrombic/antifibrinolytic activities [75, 109]. (C) To regulate the vascular tone, the endothelium releases various vasodilatory agents, such as nitric oxide (NO) and endothelial-derived hyperpolarizing factor (EDHF), or vasoconstrictive agents, such as endothelin-1 (ET-1), which modify vascular smooth muscle cell (VSMC) contractility [110]. (D) In the case of inflammation, endothelial permeability is increased. Endothelial cells recruit immune cells via the expression of adhesion molecules and mediate their transmigration toward the inner vascular wall [75, 76] .

of the atherosclerotic plaque. Radiation has been shown to upregulate several of such adhesion molecules. For instance, exposure of endothelial cells to 5 Gy increases the expression of intercellular adhesion molecule-1 (ICAM-1) and E-selectin 6 h after irradiation [111]. Platelet endothelial cell adhesion molecule-1 (PECAM-1), ICAM-1 and ICAM-2, and vascular cellular adhesion molecule-1 (VCAM-1) were also observed to increase in mouse heart cells 10 weeks after local thorax irradiation with 8 Gy [112]. Interestingly, ICAM-1 and VCAM-1 remained upregulated 20 weeks after irradiation. Besides induction of adhesion molecules, the expression of cytokines, such as interleukin (IL)-6 and IL-8, and other inflammatory molecules such as transforming growth factor-β (TGF-β) was shown to increase after high and moderate irradiation doses in human endothelial cell cultures [113, 114]. In this context, the Japanese atomic bomb survivors' cohort also showed signs of a generally increased inflammation state, with increased levels of IL-6 and C-reactive protein (CRP) [115].

3.2. DNA damage and apoptosis

Ionizing radiation is known to induce a wide range of DNA lesions, of which double-strand breaks (DSBs) are most severe in a direct manner but also indirectly through the formation of reactive oxygen species (ROS) [116, 117]. Upon DNA damage, a response is initiated, and cells activate cell cycle checkpoints that slow down or stop cell cycle progression [118]. This gives them time to repair damaged DNA or to prevent division when chromosomes are damaged or incompletely replicated. If cells fail to repair their DNA, they undergo programmed cell death, apoptosis, or premature senescence (described below) [119]. Consequently, DSB leads to a high lethality of the affected cells.

Whereas high doses are known to induce apoptosis in endothelial cells [120], less is known about the effect of low radiation doses. A subtle but significant increase in DSBs was observed in human umbilical vein endothelial cells (HUVEC) and EA.hy926 endothelial cells 30 min after exposure to 0.05 Gy. In addition, irradiation with 0.05 Gy and 0.1 Gy induced relatively more DSB/Gy in comparison to 0.5 Gy and 2 Gy [121]. This observation could be caused either by an underestimation due to DNA damage spot merging [122] or by the induction of a global chromatin reorganization at low doses of ionizing radiation [123]. Furthermore, a dose-dependent increase in the number of apoptotic cells was observed, down to 0.5 Gy in HUVEC and 0.1 Gy in EA.hy926 cells [121]. Another study showed no increase in the number of apoptotic endothelial cells after exposure to 0.2 Gy, whereas apoptosis was observed after exposure to 5 Gy [124].

3.3. Oxidative stress, mitochondrial dysfunction, and metabolic changes

Mitochondria are often regarded as the powerhouse of the cell by generating the ultimate energy transfer molecule, adenosine triphosphate or ATP. Mitochondrial dysfunction is part of both normal and premature agings, but it can also contribute to inflammation, cell senescence, oxidative stress, and apoptosis. Increasing evidence indicates that mitochondrial damage and dysfunction occur in atherosclerosis and may contribute to the multiple pathological processes underlying the disease [125].

An increased accumulation of mitochondrial DNA damage was observed in several human fibroblast cell lines after exposure to doses as low as 0.1 Gy [126]. Furthermore, functional impairment of mitochondria (reduced mitochondrial respiration and electron transport chain activity) and

alterations of the mitochondrial proteome were observed in isolated cardiac mouse mitochondria 4 and 40 weeks after a 2 Gy local heart irradiation. Only a few alterations of the mitochondrial proteome and no effect on mitochondrial function were observed with 0.2 Gy [127, 128]. Finally, alterations of energy and lipid metabolism and perturbations of the insulin/insulin growth factor—phosphatidylinositol-4,5-bisphosphate 3-kinase—RAC-alpha serine/threonine-protein kinase (IGP-PI3K-Akt) signaling pathway were suggested in proteomic studies using cell lines or cells isolated from mice after irradiation with doses ranging from 3 to 16 Gy [129–131].

Water radiolysis instantly causes the formation of ROS (e.g., $\bullet OH^-$, $\bullet O_2^-$, H_2O_2). However, cellular oxidative stress can also be observed long after irradiation, due to an increase in endogenous cellular ROS production [132]. Mitochondria are believed to be the major source of radiation-induced secondary ROS. For instance, Leach et al. have demonstrated that between 1 and 10 Gy, the amount of ROS-producing cells increased with the dose, which they suggested was dependent on radiation-induced propagation of mitochondrial permeability transition via a Ca^{2+}-dependent mechanism [133, 134]. It has further been suggested that ROS can be transferred from cell to cell by gap junctions and paracrine communication pathways in order to propagate radiation-induced biological effects at the intercellular level. This phenomenon is commonly referred to as the radiation-induced "bystander effect" [135]. Multiple molecular signaling mechanisms involving oxidative stress, various kinases, inflammatory molecules, and Ca^{2+} are postulated to contribute to this effect [136].

3.4. Premature senescence

The culprit of radiation-induced premature senescence is most likely severe irreparable DSB [137], even if accelerated telomere shortening has also been suggested [138]. Furthermore, oxidative stress is seen as a major player in radiation-induced senescence and is involved in both radiation-induced DNA damage and accelerated telomere attrition [138–140].

In several in vitro studies, it has been demonstrated that ionizing radiation induces endothelial cell senescence, mainly with exposure to higher radiation doses [141–144]. An interesting study was carried out to examine the effect of chronic low-dose rate irradiation (1.4, 2.4, and 4.1 mGy/h) during 10 weeks [145, 146]. Exposure to 1.4 mGy/h did not accelerate the onset of senescence, whereas exposure to 2.4 mGy/h and 4.1 mGy/h did. Remarkably, a senescent profile was observed when the accumulated doses received by the cells reached 4 Gy. Proteomic analysis revealed a role for radiation-induced oxidative stress and DNA damage, resulting in induction of the p53/p21 pathway. Also, a role for the PI3K/Akt/mechanistic target of rapamycin (mTOR) pathway was suggested. In a related transcriptomic study, authors suggested that premature senescence resulted from an early stress response with p53 signaling, cell cycle changes, DNA repair, and apoptosis observed after 1 week of exposure and an inflammation-related profile observed after 3 weeks. In addition, a possible role of insulin-like growth factor-binding protein 5 (IGFBP-5) signaling, known to be involved in the regulation of cellular senescence, was suggested for the induction of premature senescence after chronic low-dose rate irradiation [147].

Oxidative stress, inflammation, and cellular senescence are all consequences of a normal aging process but are observed early in irradiated tissues, including the heart, suggesting an intensification and acceleration of these molecular processes [71].

3.5. Intercellular communication

Traditionally, it was accepted that exposure to ionizing radiation only directly affects irradiated cells. However, in 1992, it was found that irradiation of 1% cells with α-particles leads to genetic damage in more than 30% of cells [148]. Exposure of cells to ionizing radiation results in significant biological effects occurring in both irradiated and non-irradiated cells through the radiation-induced bystander effect [149, 150]. Although the mechanisms of this effect are not fully elucidated yet, oxidative stress, different cytokines (e.g., TNF-α, IL-1, and IL-6), Ca^{2+}, and kinases play a role in the damage to non-irradiated cells.

Intercellular communication through gap junctions and paracrine signaling through hemichannels have been suggested to mediate bystander responses. Gap junctions and hemichannels are composed of multimeric transmembrane structures made of connexin (Cx) [150, 151]. In human, 21 Cx proteins have been identified, which are present in most organs, and display a tissue/cellular specificity [152, 153]. There are three different Cx isotypes expressed in endothelial cells of major arteries, namely, Cx37, Cx40, and Cx43 [154–156]. Cxs have important physiological roles (e.g., they support longitudinal and radial cell-cell communication in the vascular wall), and changes of their expression patterns have been observed during atherosclerosis. Although healthy endothelial cells mainly express Cx37 and Cx40, both Cxs are lost in the endothelium covering advanced atherosclerotic plaques. On the contrary, Cx43 is detectable at specific regions of advanced atherosclerotic plaques [157]. The mechanisms responsible for modification of the Cx expression pattern in atherosclerosis are not fully understood. However, it has been recently demonstrated that Cx37 is a regulator of endothelial NO synthase (eNOS) [158]. The altered Cx37 expression level could be responsible for decreased eNOS activity and decreased NO bioavailability, which may cause endothelial cell dysfunction and increased susceptibility to atherosclerosis. Therefore, Cx37 may play a protective role against atherosclerosis. In addition, Cx40 may play a similar role, as endothelial-specific deletion of Cx40 was reported to promote atherosclerosis by increasing CD73-dependent leukocyte adhesion to the endothelium [155]. In contrast to the atheroprotective effects of Cx37 and Cx40, Cx43 has been suggested to be pro-atherosclerotic. Indeed, downregulation of Cx43 expression inhibited monocyte-endothelial adhesion by decreasing the expression levels of cell adhesion proteins, whereas its upregulation enhanced the adhesion of monocytes to endothelial cells [159]. Besides their roles in atherosclerosis, Cxs were reported to be highly sensitive to ionizing radiation [156]. Indeed, it was observed that a low-dose irradiation exposure induced activation of Cx43 in a time- and dose-dependent manner in human neonatal foreskin fibroblasts [160]. Moreover, upregulation of Cx43 was noticed after 5 Gy of X-ray exposure in mouse primary endothelial cells [161].

4. Conclusion

Research regarding CVD risk related to ionizing radiation is an important way forward to complement epidemiological data with the underlying biological and molecular mechanisms. This is especially important for doses <0.5 Gy, for which epidemiological data are suggestive rather than persuasive. Indeed, due to limited statistical power, the dose-risk relationship is undetermined below 0.5 Gy, but if this relationship proves to be without a threshold, it

may have a considerable impact on current low-dose health risk estimates. In this regard, a complete understanding of the pathological effects of ionizing radiation regarding endothelial dysfunction is needed. In addition, it will help in the identification of protective strategies as well as a set of predictive biomarkers for radiation-induced cardiovascular disorders.

Acknowledgements

This review is written in the context of a study that was funded by EU FP7 DoReMi (grant agreement 249689) on "low dose research towards multidisciplinary integration,", by EU FP7 ProCardio project (grant agreement 295823) and by the Federal Agency of Nuclear Control (FANC-AFCN, Belgium) (grant agreement: CO-90-13-3289-00). R. Ramadam and B. Baselet are recipients of a doctoral SCK•CEN/Ghent University grant and of a doctoral SCK•CEN/ Université catholique de Louvain grant, respectively. P. Sonveaux is a Senior Research Associate of the Belgian National Fonds de la Recherche Scientifique.

Author details

Bjorn Baselet[1,2], Raghda Ramadan[1,3], Abderrafi Mohammed Benotmane[1], Pierre Sonveaux[2], Sarah Baatout[1,4] and An Aerts[1]*

*Address all correspondence to: an.aerts@sckcen.be

1 Radiobiology Unit, Institute for Environment, Health and Safety, Belgian Nuclear Research Centre (SCK•CEN), Mol, Belgium

2 Pole of Pharmacology and Therapeutics, Institut de Recherche Expérimentale et Clinique (IREC), Université catholique de Louvain (UCL), Belgium

3 Department of Basic Medical Sciences, Physiology Group, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium

4 Department of Molecular Biotechnology, Ghent University, Ghent, Belgium

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